

IT IS CLAIMED:

1. A set of electrophoretic tag (e-tag) probes for detecting the binding of or interaction between each or any of a plurality of ligands and one or more target antiligands, the set comprising j members, and each of said e-tag probes having the form:

(D, M_j) - L - T_j , where

(a) D is a detection group comprising a detectable label;

(b) T_j is a ligand capable of binding to or interacting with a target antiligand,

(c) L is a linking group connected to T_j by a bond that is cleavable by a selected cleaving agent when the probe is bound to or interacting with the target antiligand, wherein cleavage by said agent produces an e-tag reporter of the form (D, M_j) - L', where L' is the residue of L attached to (D, M_j) after such cleavage,

(d) M_j is a mobility modifier having a charge/mass ratio that imparts a unique and known electrophoretic mobility to a corresponding e-tag reporter of the form (D, M_j) - L', within a selected range of electrophoretic mobilities with respect to other e-tag reporters of the same form in the probe set; and

(e) (D, M_j)- includes both D - M_j - and M_j - D -;

said uncleaved or partially cleaved e-tag probes, but not the corresponding e-tag reporter, having one or more chemical groups capable of reacting with or binding to a selected capture agent that is effective to

(i) impart a mobility to the probes bound to capture agent that prevents the probes from electrophoretically migrating within said range of electrophoretic mobilities or

(ii) immobilize the probes on a solid support.

2. The probe set of claim 1, for detecting each or any of a plurality of known, selected target nucleotide sequences, the set comprising j members, wherein:

(a) T_j is an oligonucleotide target-binding moiety having a sequence of nucleotides U_i connected by intersubunit linkages $B_{i,i+1}$, where i includes all integers from 1 to n , and n is sufficient to allow the target-binding moiety to hybridize specifically with a target nucleotide sequence;

(b) L is a nucleotide joined to U_1 in T_j through a nuclease-cleavable bond; and

(c) each of the target-binding moieties contains at least one modification selected from the following:

(i) at least one nuclease-resistant bond $B_{i,i+1}$, where i includes at least 1;

(ii) U_1 containing a capture ligand capable of binding specifically to a capture agent; and

(iii) a nuclease-resistant bond $B_{i,i+1}$, where i includes at least 1, and at least one nucleotide U_i containing a capture ligand capable of binding specifically to a capture agent, where $i \geq 1$.

3. The probe set of claim 1, wherein L includes at least a portion of an amino acid

sequence that is recognized and cleaved by a selected peptidase.

4. The probe set of claim 1, wherein L includes at least a portion of an oligosaccharide that is recognized and cleaved by a selected hydrolytic enzyme.

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5. The probe set of claim 1, wherein L and T_j are linked by an ester linkage that is cleaved by a selected esterase.

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6. The probe set of claim 1, wherein L and T_j are linked by a disulfide bond, and the antiligand is attached to an oxidase enzyme, such that in the presence of a substrate for the enzyme, H₂O₂ generated by the oxidase is effective to cleave the disulfide linkage in a probe bound to the antiligand.

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7. The probe of claim 1, wherein L and T_j are linked by a bond cleavable by singlet oxygen, wherein the antiligand is attached to a sensitizer capable of generating singlet oxygen when photoactivated.

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8. The probe set of claim 1, for use in detecting the binding of each or any of a plurality of ligands to a target antiligand molecule, wherein the plurality of ligands are represented by T_j.

How is this limiting to Claim 1?

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9. The probe set of claim 1, for use in screening for a ligand capable of binding to a receptor, wherein the ligands are represented by T_j and are members of a combinatorial library of small organic molecules, and the antiligand is the receptor.

NOTE: This claim was originally written as a dependent claim to Claim 7 above.

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10. The probe set of claim 1, for use in screening substrates of a selected enzyme antiligand, wherein the substrate comprises a fixed moiety L and a variable moiety T_j, and interaction of a substrate probe with the enzyme is effective to cleave the substrate to release the T_j moiety from the substrate.

NOTE: This claim was originally written as a dependent claim to Claim 7 above.

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11. The probe set of claim 1, wherein each M_j has a unique charge/mass ratio by virtue of variations in mass, but not charge.

12. The probe set of claim 1, wherein each M_j has a unique charge/mass ratio, by virtue of changes in both mass and charge.

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13. The probe set of claim 12, containing at least 5 probes whose corresponding e-tag

reporters have unique charge/mass ratios of between -0.001 and 0.5.

NOTE: The 033.00US spec (page 10, line 23) cited a "range of about -0.0001 to 0.1, usually in the range of about -0.001 to about 0.5." The 0.1 was thought to be in error.

5 14. The probe set of claim 12, containing at least 9 probes whose corresponding e-tag reporters have unique charge/mass ratios of between -0.001 and 0.5.

15. The probe set claim 12, wherein each M_j is formed of a selected number of negatively charged and/or positively charged amino acids.

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16. The probe set of claim 12, wherein each M_j includes an alkyl chain, and differs from other M_j in the set by 1-3 methylene groups in the chain.

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17. The probe set claim 1, wherein the detectable label is selected from the group consisting of a fluorophore, a chromophore, and an electrochemical compound capable of a detectable reaction in the presence of a redox agent.

18. The probe set of claim 1, wherein the detectable label has a selected mass and charge.

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19. The probe set of claim 18, containing subsets of probes, each subset having a label with a unique mass/charge ratio.

20. The probe set of claims 18 and 19, wherein the detectable label is a fluorophore.